is prone to drop into ADP inhibition which is a common regulation mechanism to prevent wasteful ATP hydrolysis when an electrochemical ion gradient across the membrane is not available. The soluble portion F1 is capable of ATP hydrolysis, accomplished by the minimum catalytic core subunits $\alpha_3\beta_3\gamma$. A special feature of cyanobacterial and chloroplast F1 is an inserted amino acid sequence in the γ-subunit. In chloroplasts, the insertion is slightly extended and additionally contains two cysteines thus enabling thiol modulation. This higher plant-specific molecular switch was transferred to a chimera F1 by inserting the small cysteine-containing chloroplast sequence into a cyanobacterial γ-subunit [1]. Reduction of the disulfide bridge elevates latent ATPase and might represent a mechanism to prevent wasteful ATP hydrolysis in the dark. Although there are indications that suppression of ATP hydrolysis under oxidizing conditions involves ADP inhibition properties [1], the propagation of structural information between the site of thiol modulation in the γ-subunit and the catalytic sites in the β-subunits remains unclear. Here, a chimera F1 redox mutant study concluded a critical interplay between the γ-subunit "neck" region and the turn region of the βDELSEED-loop (i.e. βI397 and βL398, ATP synthase of *Thermosynechococcus elongatus* BP-1 numbering). Several mutants were designed with non-effective, weakened, enhanced and reversed thiol modulation. At selected positions of putative interactions, redox regulation of ATP hydrolysis appeared to be affected by substituent properties such as hydrophobicity and bulkiness. Removal of charged residues within the βDELSEED motif suggested that ionic interactions with the γ-subunit play a less important role in processing thiol modulation. For the first time, β-subunit residues were assigned to receive information produced by disulfide/dithiol formation in the γ-subunit. [1] Y. Kim, H. Konno, Y. Sugano, T. Hisabori, Redox regulation of rotation of the cyanobacterial F1-ATPase containing thiol regulation switch, J. Biol. Chem. 286 (2011) 9071–9078.

doi:10.1016/j.bbabio.2014.05.197

S1.P5

Generation of a mitochondrial membrane potential in the absence of mtDNA: Can we obtain fresh insight by going back to the root?

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Increasing evidence places the root of the eukaryotic tree of life between the supergroup Excavata and all other eukaryotes, making these organisms important subjects to study fundamental aspects of mitochondrial biology and bioenergetics. The excavate parasite *Trypanosoma brucei* shows unusual and fascinating mitochondrial features, including a gigantic organellar DNA network (the kinetoplast or kDNA) and extensive posttranscriptional mRNA editing. As *T. brucei* switches between insect and mammalian hosts, mitochondrial function and energy metabolism undergo dramatic transformation. In insect stage parasites, oxidative phosphorylation requires FoF1-ATP synthase to generate ATP. In the bloodstream stage the enzyme operates as a proton pump to maintain the essential mitochondrial membrane potential, ΔΨm. In *T. brucei*, ATP synthase subunit 6 (a), an Fo subunit critical for proton transport, is kDNA-encoded, but trypanosomes can exist without kDNA due to specific mutations (e.g. L262P) in the nuclearly-encoded gamma subunit of F1 [1], a situation reminiscent of petite-negative yeast [2]. In yeast and mammalian cells, survival of mtDNA loss is proposed to use an alternative mode for generating ΔΨm that involves the ADP/ATP carrier and F1 [2], but the precise mechanism remains unclear. We use kDNA0 bloodstream form *T. brucei* to investigate: 1) The consequences of the gamma mutation on FoF1 structure, 2) the molecular mechanism of compensation for kDNA loss, and 3) the consequence of kDNA deletion on life cycle progression. High resolution clear native electrophoresis shows dramatic consequences to FoF1 structure in the presence of an L262P mutation. Cells show reduced or abolished expression of subunit 6 and oligomycin resistance. The apparent selection for cells that no longer express intact FoF1 suggests that L262P uncouples the enzyme, resulting in a lethal proton leak. Differentiation studies demonstrate that cells do not require kDNA for formation of transmissible intermediate stages, but that kDNA is indispensable for progression to the insect form. [1] S. Dean, M.K. Gould, C.E. Dewar, A.C. Schnaufer, Single point mutations in ATP synthase compensate for mitochondrial genome loss in trypanosomes, Proc. Natl. Acad. Sci. U.S.A., 110 (2013) 14741–14746. [2] X.J. Chen, G.D. Clark-Walker, Specific mutations in alpha- and gamma-subunits of F1-ATPase affect mitochondrial genome integrity in the petite-negative yeast Kluyveromyces lactis, EMBO J, 14 (1995) 3277–3286.

doi:10.1016/j.bbabio.2014.05.198

S1.P6

Dimer and monomer of F1F0-ATP synthase in *Ustilago maydis*: Structural composition

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The F1F0-ATP synthase produces more than 90% of cellular ATP coupled to the proton electrochemical potential. This enzyme consists of two major sectors, the sector F0 which contains the c9–10, a and b subunits, and is powered by the proton electrochemical potential; and the soluble and catalytic sector F1, containing the alpha, beta, gamma, delta and epsilon subunits. In yeast and mammalian mitochondria, F0 contains an additional protein called OSCP [1–2]. In the last years, complex V has been described as a homo-dimer (V2), which plays a role in the architecture of the mitochondrial cristae; particularly, V2 is recognized as the main entity involved in the structure of the crista tips. Actually, the identity of the dimerizing proteins suggests that this is species-specific. Although the knowledge about V2 composition and stoichiometry and its role in the crista architecture has been studied, little is known about the kinetic behavior of this homo-oligomer. In this work, the dimer (V2) and the monomer (V1) of the mitochondrial F1F0-ATP synthase from the dimorphic basidiomycete *Ustilago maydis* were studied. The V1 and V2 were solubilized with the soft detergent digitonin and isolated using a sucrose gradient (1.5 to 0.5 M), and their subunit composition obtained by MS/MS. The V2 contained all V1 subunits, plus subunits e, g and h. Analysis of the sequences of the subunits shows that alpha subunit was shorter than beta subunit. However the alpha gene had similar length than their homologues; this suggested a post-translational modification of this subunit without affecting its ATP hydrolysis activity. Additionally, the prohibitins (PHB1 and PHB2) and ADP/ATP translocase were identified in both oligomeric forms of the F1F0-ATP synthase. [1] F. Minauro-Sanmiguel, S. Wilkens, J.J. García, Structure of dimeric mitochondrial ATP synthase: novel F0 bridging features and the structural basis of mitochondrial cristae biogenesis, PNAS. 102 (2005) 12356–12358. [2] S. Couoh-Cardel, S. Uribe-Carvajal, S. Wilkens, J.J. García-Trejo, Structure of dimeric F1F0-ATP synthase, The Journal of biological chemistry. 285 (2010) 36447–36455.

Acknowledgement

This work was supported by Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (IN214914, IN209614) from Universidad Nacional Autónoma de México (UNAM). Mercedes

Esparza-Perusquía is a PhD student of the Ciencias Biológicas (511021118) of Universidad Nacional Autónoma de México and fellow to CONACYT (254400).

doi:10.1016/j.bbabio.2014.05.199

S1.P7

The role of residue beta249 in MgADP inhibition of *Escherichia coli* ATP synthase

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When MgADP without phosphate is bound at a catalytic site of H^+-FoF1 ATP synthase, there is a probability that the enzyme gets blocked in an inactive conformation. This phenomenon is known as MgADP inhibition. Earlier experiments indicate that the enzyme adopts a special inactive conformation with MgADP trapped in a catalytic site. This is a core feature of ATP synthase observed even in the minimal catalytic alpha3beta3gamma-complex. MgADP inhibition is found in all ATP synthases studied so far. However, the degree of inhibition varies considerably, being strong in mitochondria and especially chloroplast enzyme, as well as in some bacteria, e.g. *Bacillus* PS3, but rather weak in FoF1 from *Escherichia coli*. We have demonstrated previously that in *Bacillus* PS3 ATP synthase mutation betaGln259Leu can dramatically decrease MgADP inhibition. In *E. coli* wild type enzyme the residue in the corresponding position (beta249) is Leu, i.e. it is similar to the mutant *Bacillus* PS3 enzyme. In this work we made an *E. coli* mutant betaLeu249Gln. The mutation significantly enhanced MgADP inhibition. Moreover, it completely changed the effect of phosphate on MgADP inhibition, while in the wild-type enzyme phosphate increased the inhibitory effect of MgADP, and in the betaLeu249Gln it relieved the inhibition. We conclude that MgADP inhibition is a relatively easily modulated regulatory feature of ATP synthase rather than an inevitable "side effect" of the rotary catalytic mechanism.

doi:10.1016/j.bbabio.2014.05.200

S1.P8

The Fo complex of the chloroplast ATP synthase — Towards a structural study of a macromolecular nanomotor

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Powered by the proton-motive force (pmf) of the cell, F-type ATP synthases are the major producers of adenosine triphosphate (ATP) in cells. The ATP synthase in plant chloroplasts consists of two domains, the soluble F1 and the membrane-embedded Fo subcomplex. In Fo the pmf across the thylakoid membrane is converted into torque and induces rotation of a rotor element, the c-ring. The unique construction of the Fo complex allows ions (H^+ or Na^+) to pass a distinct pathway through the rotor–stator elements and surmount the membrane from one side to another. The rotation is tightly coupled with F1 and elicits conformational changes in the catalytic beta-subunits, finally leading to ATP synthesis. While the function of the F1 complex and the Fo rotor element has unveiled by many high-resolution structures in the last 20 years, structural information about the complete Fo complex or its neighboring stator subunits a and b is still scarce. To understand the molecular mechanism of this nanomotor, high resolution structural information is indispensable. We set out to investigate the chloroplast Fo-ATP synthase complex by structural methods. By combining classical biochemical purification strategies such as sucrose density centrifugation and ion-exchange chromatography we were able to establish a protocol for a large scale purification of the ac14 sub-complex of the *Spinacia oleracea* ATP-synthase. The isolation of abundant and stable ac14 allows subsequent experiments such as 2D and 3D crystallization and their structural characterization by electron microscopy and X-ray crystallography.

doi:10.1016/j.bbabio.2014.05.201

S1.P9

A theoretical treatment of energy transfer in a molecular motor based on Brownian motion on a multidimensional free-energy landscape: The derivation of formal analytic results in the Kramers regime of deep potential wells

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In a cell, specialised proteins act as molecular-scale motors to convert energy from one form to another. For example, ATP synthase converts chemical energy into mechanical work and back again, ion pumps convert chemical energy into transport against an electrochemical gradient, and cytoskeletal motors use chemical energy to transport loads along linear molecular chains. Despite functional differences between different types of molecular motors, there are fundamental similarities in their behaviour and properties: i) they transfer energy between different degrees of freedom (i.e., chemical and mechanical), ii) they are subject to large thermal fluctuations, and iii) they operate far from equilibrium. We present a general theoretical treatment of energy transfer in a molecular motor based on Brownian motion on a multidimensional free-energy landscape. This treatment enables energy transfer between degrees of freedom to be described explicitly and captures key fundamental elements of molecular motor operation. Unfortunately, the multidimensional diffusion equation arising in this treatment is not solvable in general. We transform the continuous diffusion equation to a simpler discrete master equation that is analytically tractable [1]. This provides an opportunity to connect the general continuous multidimensional Brownian-motion based theory with experiments, phenomenological models, and established results from non-equilibrium thermodynamics. In the Kramers regime of deep potential wells, we derive a range of formal expressions for molecular motors. We determine physical properties including the drift and diffusion, the rate and efficiency of energy transfer, and the entropy generated [2]. We also consider thermal fluctuations for the motor and show that energy transfer between degrees of freedom creates statistically correlated fluctuations in those degrees of freedom [3]. The fluctuation statistics provide an opportunity to distinguish different operating regimes of the motor.

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